

A FINE STRUCTURE STUDY OF LIPID IN MOUSE LIVER REGENERATING AFTER PARTIAL HEPATECTOMY

NANCY L. TROTTER, Ph.D.

From the Department of Anatomy, Columbia University College of Physicians and Surgeons, New York

ABSTRACT

The fine structure of liver 3½ to 72 hours after partial hepatectomy has been compared with that of liver from sham-operated animals; all animals were 60- to 90-day old male mice of the C3H strain. Numerous small bodies with diameters ranging from 300 to 1,000 Å have been observed distributed randomly throughout the cytoplasm of the hepatic parenchymal cells at early intervals after partial hepatectomy. In material fixed in osmium tetroxide and embedded in methacrylate, they appear as uniformly electron-opaque bodies, but in permanganate-fixed liver, they display only a peripheral rim of electron-opaque material surrounding a clear core. Each of these cytoplasmic bodies appears to be located within a vesicle. A few of the opaque bodies are also present in sinusoids and in the spaces of Disse; these bodies are not located within vesicular structures. Fat droplets of various sizes are easily distinguished in regenerating liver; with the increase in number of these fat droplets at later postoperative intervals, there occurs a concomitant decrease in the number of cytoplasmic bodies. It is suggested that the cytoplasmic bodies contain some lipid component. Possible explanations of the origin, nature, and fate of the cytoplasmic bodies are discussed.

INTRODUCTION

The cytology and biochemistry of liver regenerating after partial hepatectomy has been studied extensively (1-13), and much information is available concerning the behavior of the various cell constituents during regeneration. Among the investigators of regenerating liver, there seems to be universal agreement concerning an early accumulation of fat following partial hepatectomy. Yokoyama *et al.* (10), using strain A mice, found that a considerable amount of fat is present during the first 3 days following partial hepatectomy; they further demonstrated that glycogen depots are depleted at this time, but that glycogen is re-deposited later at the periphery of newly formed

daughter cells. In rats, as demonstrated histochemically by Takahashi (14), there is marked decrease in the number of fat droplets 3 days after partial hepatectomy; an increased glycogen content was observed at 48 hours and persisted through 3 days.

With the application of electron microscopy to the study of liver regeneration following partial hepatectomy, various interesting phenomena have been uncovered. In 1952, Bernhard *et al.* (15) observed enlarged nuclei and nucleoli and an increased amount of granular endoplasmic reticulum in rat liver 48 hours after partial hepatectomy. Later, Rouiller and Bernhard (16)

reported an increase in the number and size of microbodies in rat liver. In 1960, Takahashi (14) noted the appearance of fat droplets, the disappearance of ribosomes from the endoplasmic reticulum, and the swelling of mitochondria in regenerating rat liver at early postoperative intervals.

Since much histochemical and biochemical information has been accumulated concerning the regenerating liver, examination of the fine structure of an organ in such an active state of synthesis should offer an opportunity for the study of possible relationships between cytoplasmic components and certain of the known physiological processes of the cell. In this study, emphasis is placed on the physiological significance of the appearance and disappearance of cytoplasmic bodies and fat droplets following partial hepatectomy.

MATERIALS AND METHODS

Twenty-six male mice of two sublines of the C3H strain (C3H/StWi and C3H/AnWi) were used in this study. All animals were between 60 and 90 days of age; they were maintained at 78°F. and were allowed Purina Laboratory Chow and water *ad libitum*.

Partial hepatectomy was performed according to the method of Higgins and Anderson (17), and animals were sacrificed at 3½, 7, 11½, 16, 24, 48 and 72 hours after operation. All experimental animals were sacrificed, with sham-operated controls, at the same time of day (midmorning) in order to avoid possible variation due to the existence of any diurnal rhythm such as that exhibited by mitotic activity and glycogen content (18, 19). Liver which was fixed in 2 per cent osmium tetroxide buffered with Veronal-acetate was embedded in methacrylate and stained with neutral lead acetate (20). Potassium permanganate, which is insoluble in fat (21), was also employed as a fixative; material so prepared was embedded in methacrylate or Epon 812 and examined unstained, stained with neutral lead acetate, with basic lead acetate (22), or with lead citrate (23). Liver was also fixed in glutaraldehyde, refixed in osmium tetroxide, embedded in Epon 812, and stained as was the permanganate-fixed material; in glutaraldehyde preparations refixed in osmium tetroxide, particulate glycogen is rendered visible (24). Sections were cut on a Porter-Blum microtome and examined in RCA EMU 3C, D, F, and G electron microscopes. Light microscope studies were carried out on preparations fixed in formalin, Bouin's fluid, or Rossman's fluid, and stained with Sudan IV, hematoxylin and eosin, or periodic acid-Schiff reaction.

OBSERVATIONS

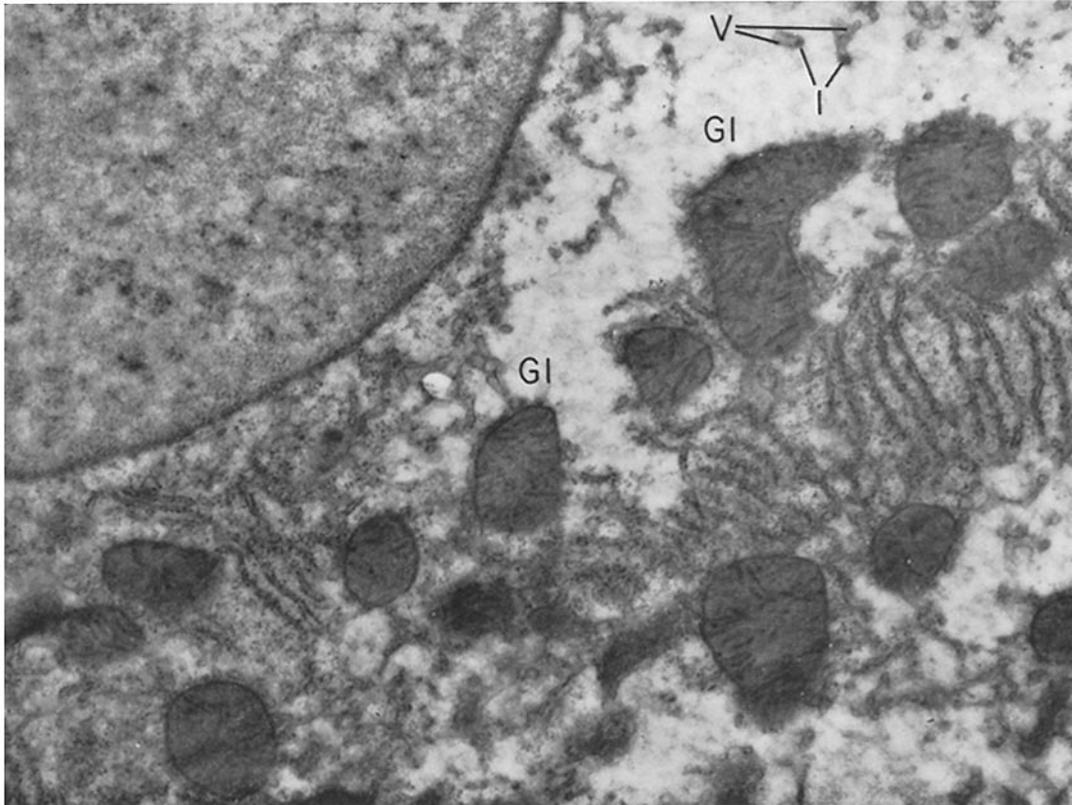
Liver after Sham-Operation

The fine structure of liver after sham-operation is similar to that described elsewhere (25-27) for the liver of normal mice of other strains and for rats. In preparations fixed in Veronal acetate-buffered osmium tetroxide, glycogen areas are recognizable as large well defined areas within the cytoplasm; particulate glycogen is visible in material fixed in phosphate-buffered osmium (28) or in material fixed in glutaraldehyde and refixed in osmium (24), but cannot be distinguished in preparations fixed in Veronal-acetate-buffered osmium tetroxide. A few fat droplets are present, usually located within glycogen areas, and are occasionally seen in close association with mitochondria. After sham-operation, a few extremely minute electron-opaque bodies can occasionally be observed in the cytoplasm of parenchymal cells of livers which have been fixed in osmium tetroxide (Fig. 1). Each of these minute bodies appears to be enclosed within a vesicle. Examination of control tissues with the light microscope discloses the presence of a large amount of glycogen and a few fat droplets.

Liver Regenerating after Partial Hepatectomy

Many differences in fine structure exist between liver from sham-operated animals and liver regenerating after partial hepatectomy. At 3½ hours after partial hepatectomy, alterations in the liver are already apparent and continue to be observed through 48 hours. In general, the change which occurs is a gradual one, since differences in the fine structure of liver of animals sacrificed at intervals more widely separated from one another are greater than the differences observed in the liver of animals sacrificed at closer intervals.

The most conspicuous change in regenerating liver concerns the appearance of numerous cytoplasmic bodies ranging from 300 to 1,000 Å in diameter. These bodies are considerably larger and more numerous than those observed in liver after sham-operation. In material fixed in osmium tetroxide, these bodies appear electron-opaque. Although they are most numerous randomly distributed throughout the cytoplasm of hepatic parenchymal cells (Figs. 2 and 3), they are also present in the spaces of Disse and in sinusoids (Fig. 4). As early as 3½ hours after partial hepatectomy (Fig. 2) the number of cytoplasmic



Unless otherwise stated, all illustrations are of liver fixed in Veronal-acetate buffered-osmium tetroxide, embedded in methacrylate, and stained with lead acetate.

FIGURE 1 Parenchymal cell from the liver of a C3H mouse $3\frac{1}{2}$ hours after sham-operation. Note the glycogen area (Gl) in which are located a few minute electron-opaque bodies (I). Each body is contained within a vesicle (V). $\times 24,500$.

bodies which can be observed is extremely high; at 7 hours the number has increased even more (Fig. 3), but by $11\frac{1}{2}$ hours it appears to drop to a level approximating that at $3\frac{1}{2}$ hours. At 16 hours after partial hepatectomy, the number of cytoplasmic bodies in regenerating liver is diminished even further, and by 24 hours they are no longer in evidence. In material fixed in potassium permanganate, the cytoplasmic bodies are not uniformly electron-opaque as they are in osmium tetroxide-fixed material, but exhibit only a peripheral rim of dense material surrounding a clear core. Each of the bodies within the cytoplasm of the parenchymal cell appears to be located within a vesicle (Figs. 2 to 5), but those in the spaces of Disse and in sinusoids have not been observed in association with any vesicular

structure (Fig. 4). The relationship of the vesicles to their enclosed cytoplasmic bodies is extremely variable (Fig. 5). Usually the vesicle is only a bit larger than the cytoplasmic body and looks almost like a limiting membrane (Fig. 5, A); in a few cases, the vesicle is much larger than the enclosed body (Fig. 5, B) and may even contain two or more cytoplasmic bodies (Fig. 5, C). Vesicles containing cytoplasmic bodies have also been observed apparently fusing (Fig. 5, D). In some instances the cytoplasmic bodies have been observed touching the outer membranes of mitochondria (Fig. 6), and in other cases they exhibit involvement with the Golgi complex (Fig. 3).

The number of fat droplets in regenerating liver is also subject to change. Light microscope observations of Sudan IV-stained preparations

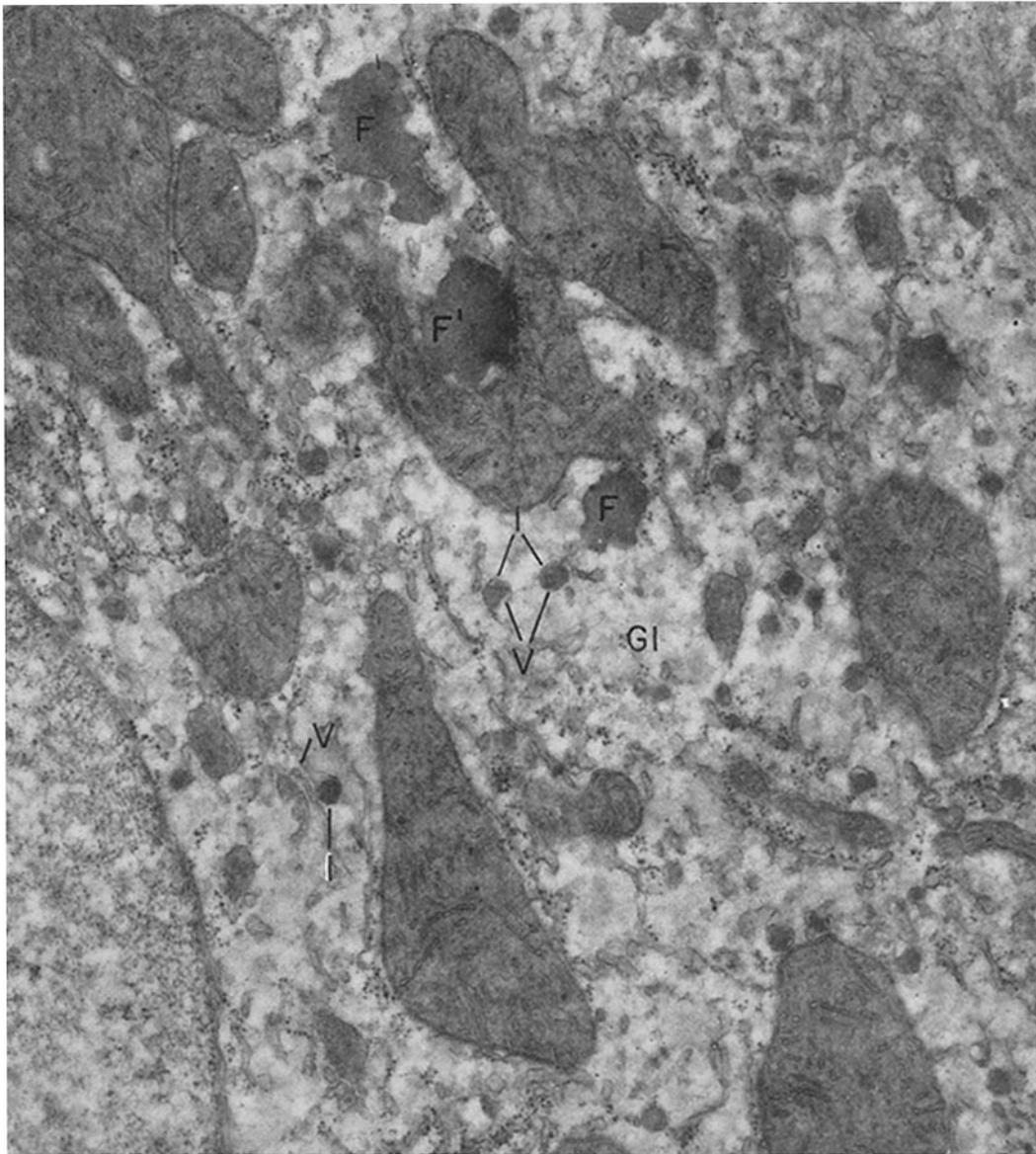


FIGURE 2 Section of a parenchymal cell from C3H mouse liver $3\frac{1}{2}$ hours after partial hepatectomy. Electron-opaque bodies (*I*) located within vesicles (*V*) may be seen randomly distributed throughout the cytoplasm. Note that the size and number of these bodies is greater than in control liver (Fig. 1). A few fat droplets (*F*) are present, one of which (*F'*) can be seen in contact with a mitochondrion. There are fewer glycogen areas (*GI*) than in control liver, but more than in liver at 7 hours after partial hepatectomy (compare with Fig. 3). $\times 32,000$.

of liver at $3\frac{1}{2}$ to 48 hours following partial hepatectomy reveal an increase in total lipid content when compared with normal or sham-operated liver. In osmium tetroxide-fixed material,

fat droplets appear extremely opaque and are usually chattered (Fig. 7). In permanganate-fixed material, the fat droplets are distinguishable by a large clear core and only a tiny rim of dense

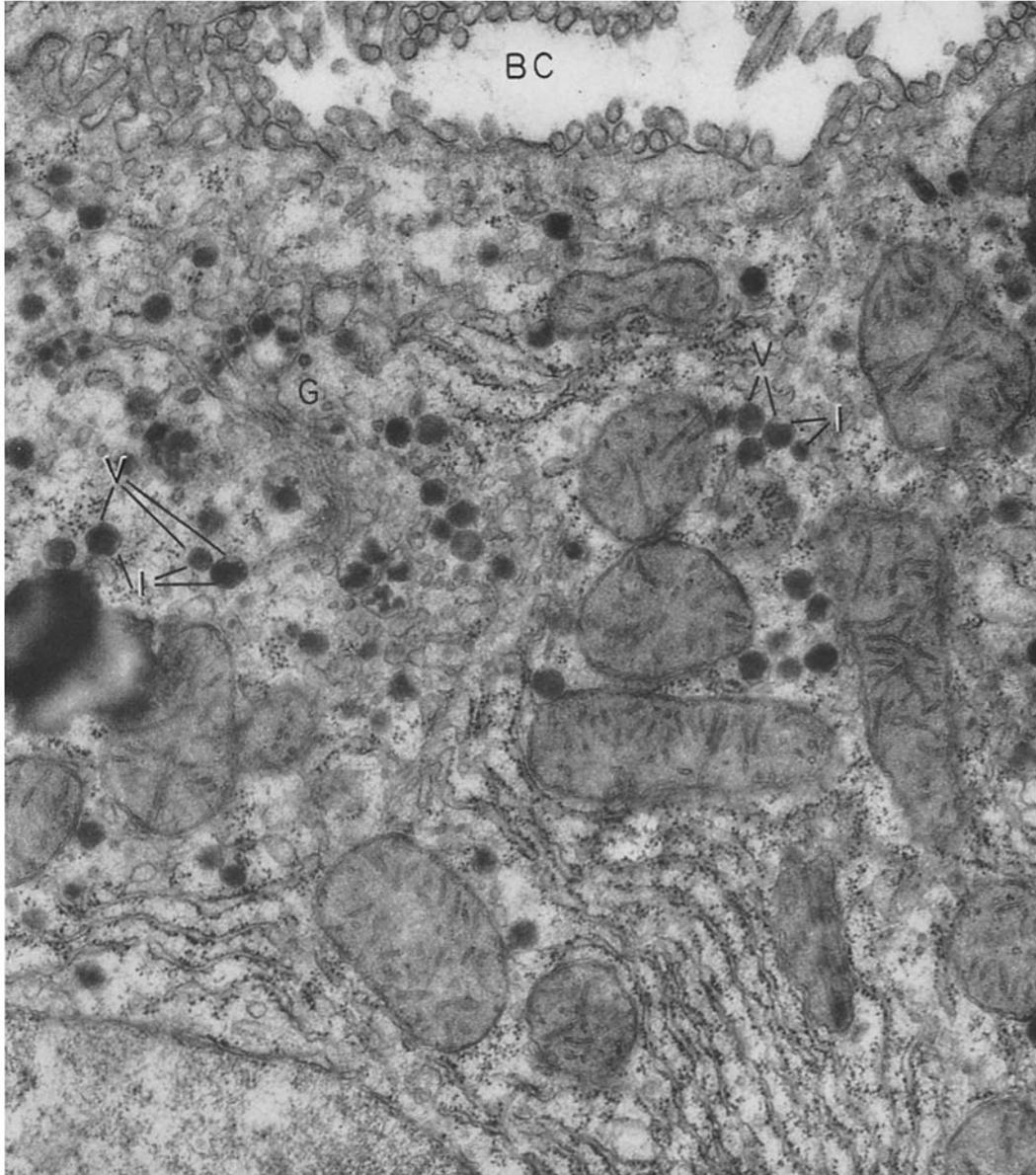


FIGURE 3 Hepatic parenchymal cell from the liver of a C3H mouse 7 hours after partial hepatectomy. Many more cytoplasmic bodies (*l*) are present than at all other intervals observed; characteristically, each body is located within a vesicle (*V*). Some of these bodies may be seen in association with the Golgi complex (*G*). In contrast to Fig. 2 ($3\frac{1}{2}$ hours after partial hepatectomy), no definite glycogen areas are present. A bile canaliculus (*BC*) is included in this section. $\times 31,500$.

material. At $3\frac{1}{2}$ and 7 hours after partial hepatectomy, the number of fat droplets in regenerating liver is only slightly greater than in control liver but increases to attain a maximum between 16

and 48 hours. Thereafter, the number of fat droplets diminishes and by 72 hours has dropped to the level of control liver. The size of the fat droplets in regenerating liver varies at all of the

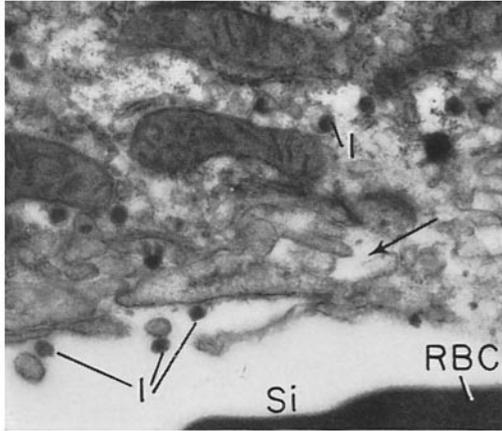


FIGURE 4 Section of a hepatic parenchymal cell, the space of Disse (arrow), and a sinusoid (Si) from the liver of a C3H mouse $3\frac{1}{2}$ hours following partial hepatectomy. A portion of a red blood cell (RBC) located in the sinusoid is included. Electron-opaque bodies (I) are present in the sinusoid, the space of Disse, and within the hepatic parenchymal cell. Note that those bodies which are located within the hepatic parenchymal cell are located within vesicles, whereas those located in the sinusoid and in the space of Disse are not. $\times 24,000$.

intervals studied after partial hepatectomy; in general, however, they appear larger after 24 than after $3\frac{1}{2}$ hours. Fat droplets apparently fusing with one another are also evident (Figs. 7 and 8). The cytoplasmic bodies may sometimes be seen apparently attached to a fat droplet (Fig. 8), and fat droplets touching mitochondria (Figs. 2 and 9) are present in all regenerating livers studied.

Changes in the glycogen content of the cells of regenerating liver are also apparent. Light microscope observations of periodic acid-Schiff preparations of regenerating liver at the early intervals ($3\frac{1}{2}$ to 16 hours) after partial hepatectomy reveal a marked decrease in glycogen content when compared to normal liver and liver of sham-operated animals. When viewed in the electron microscope, sections of liver fixed in Veronal-acetate-buffered osmium tetroxide contain fewer glycogen areas at $3\frac{1}{2}$ hours after partial hepatectomy (Fig. 2) than does control liver. At 7 hours after partial hepatectomy, no large glycogen areas can be observed either in osmium tetroxide-fixed, methacrylate-embedded material or glutaraldehyde-fixed, osmium tetroxide-refixed material embedded in Epon (Figs. 3 and 10). By $11\frac{1}{2}$

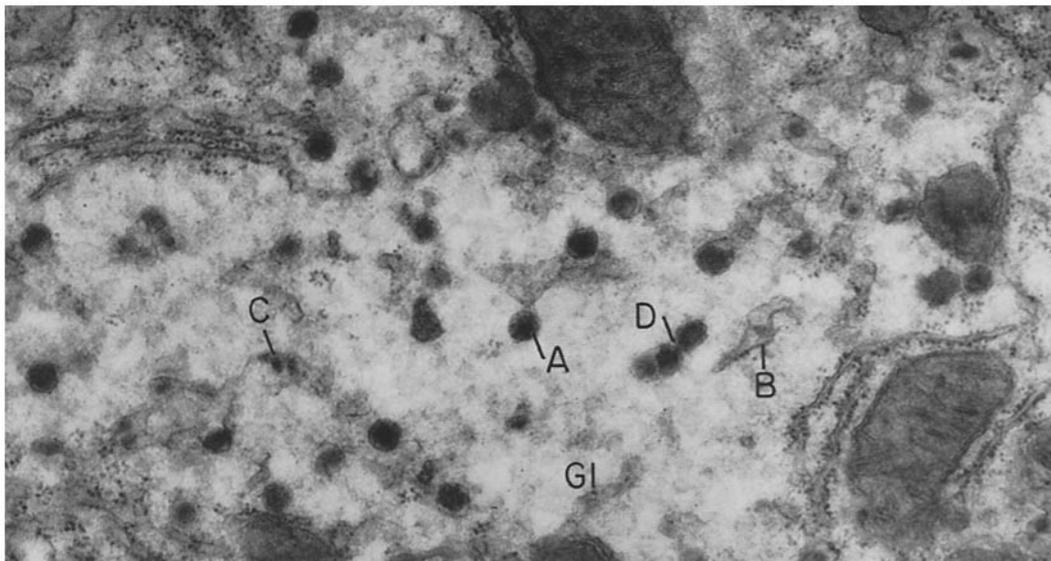


FIGURE 5 Regenerating C3H mouse liver $3\frac{1}{2}$ hours after partial hepatectomy. This shows a glycogen area (G1) in which several cytoplasmic bodies are located. Some of the vesicles are almost the same size as the cytoplasmic bodies they contain (A); other smaller bodies do not nearly fill the vesicle (B). More than one body may be seen within a single vesicle (C), and some of the vesicles are in contact (D). $\times 30,500$.

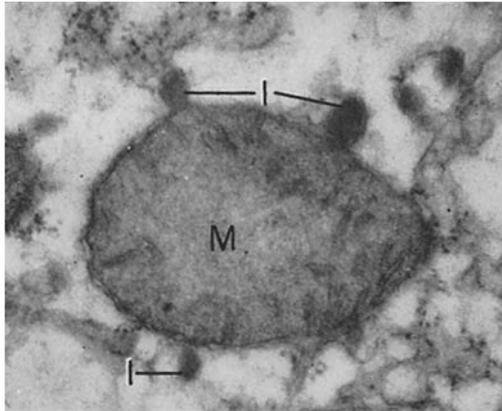


FIGURE 6 A portion of the cytoplasm of a parenchymal cell from the liver of a C3H mouse 16 hours after partial hepatectomy. The close association of cytoplasmic bodies (*I*) with a mitochondrion (*M*) can be seen. Also note the two bodies within one vesicle. $\times 44,000$.

hours a few glycogen areas have reappeared, and at 16 hours the size and number of glycogen areas have increased even further. Between 24 and 72 hours after partial hepatectomy, glycogen areas appear essentially the same as in control liver.

DISCUSSION

Numerous cytoplasmic bodies have been observed in regenerating liver at early intervals after partial hepatectomy. The exact composition of these bodies is not known; but it has been pointed out that, in osmium tetroxide-fixed material, both fat droplets and cytoplasmic bodies are extremely electron opaque; in permanganate-fixed material, cytoplasmic bodies, as well as lipid droplets, display dense peripheries and clear cores. Because of these similarities between cytoplasmic bodies and fat droplets, it is suggested that the cytoplasmic bodies contain at least some component of lipid. Other investigators have reached similar conclusions: Casley-Smith (29) has called structures,

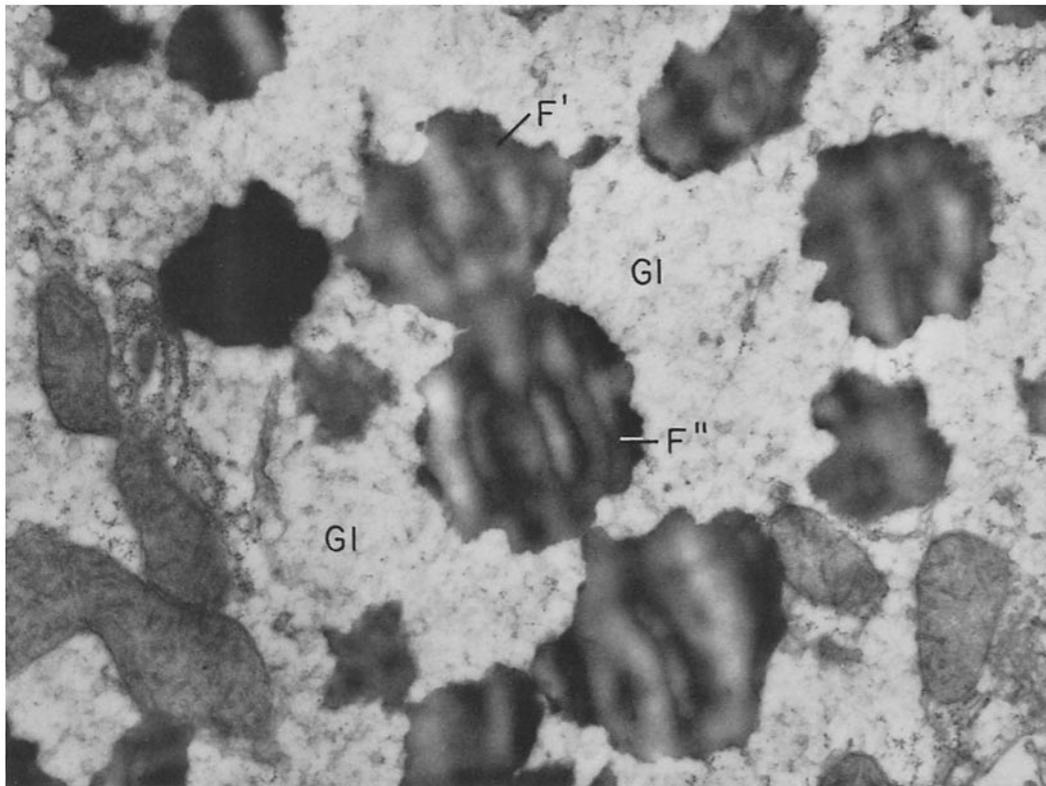


FIGURE 7 Regenerating C3H mouse liver 16 hours following partial hepatectomy. Many chattered fat droplets are located in a glycogen area (*GI*). Two of these (*F'* and *F''*) are apparently fusing. $\times 20,000$.

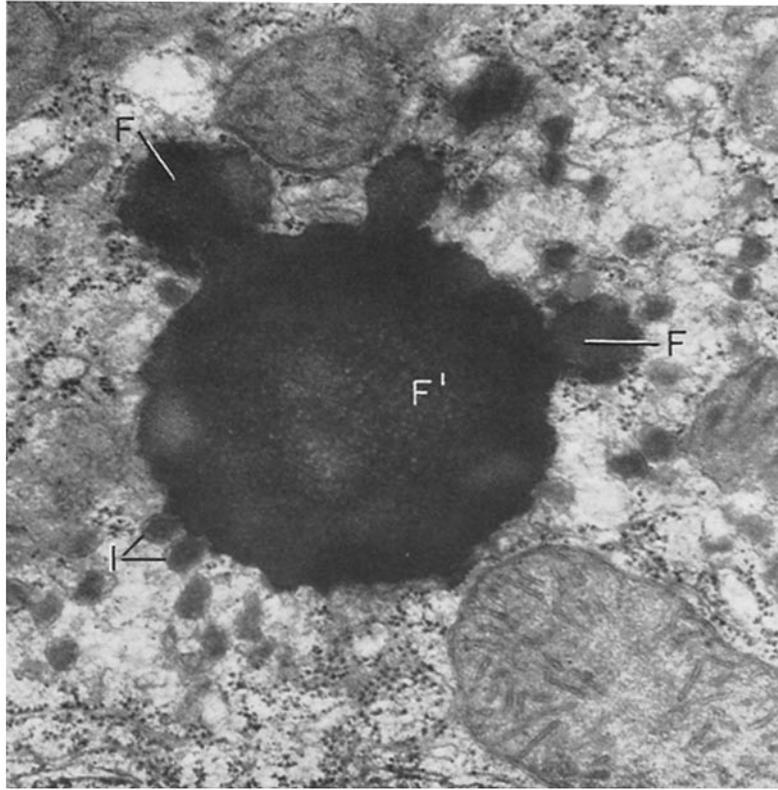


FIGURE 8 Hepatic parenchymal cell from a C3H mouse 3½ hours after partial hepatectomy. Cytoplasmic bodies (*I*) and fat droplets of various sizes (*F*) are apparently in contact with the largest fat droplet (*F'*) in the field. $\times 43,000$.

which have the same size range and fixation properties as the bodies observed here, lipoproteins; Wassermann and McDonald (30) have described similar structures as lipomicrons which, they suggest, represent lipids in transit to or from fat depots.

If the cytoplasmic bodies of regenerating liver are, in fact, lipid-containing bodies, it is still not known whether they represent an increased intake of lipid into the cell or an increased rate of *de novo* synthesis by the liver. It is known that, during starvation, fat is mobilized into the liver. Harkness (31) contends, the effects of partial hepatectomy merely represent an exaggeration of the effects of starvation. In this connection, it should be pointed out that, during the first 24 hours after partial hepatectomy, mice eat very little; moreover, when livers of fasted, non-operated animals are examined in the electron microscope, the presence of a few

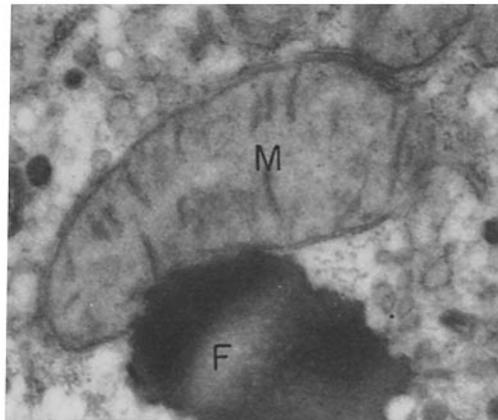


FIGURE 9 Cytoplasm of a parenchymal cell from C3H mouse liver 11½ hours after partial hepatectomy. The intimate relationship of a fat droplet (*F*) and a mitochondrion (*M*) is depicted. $\times 35,000$.

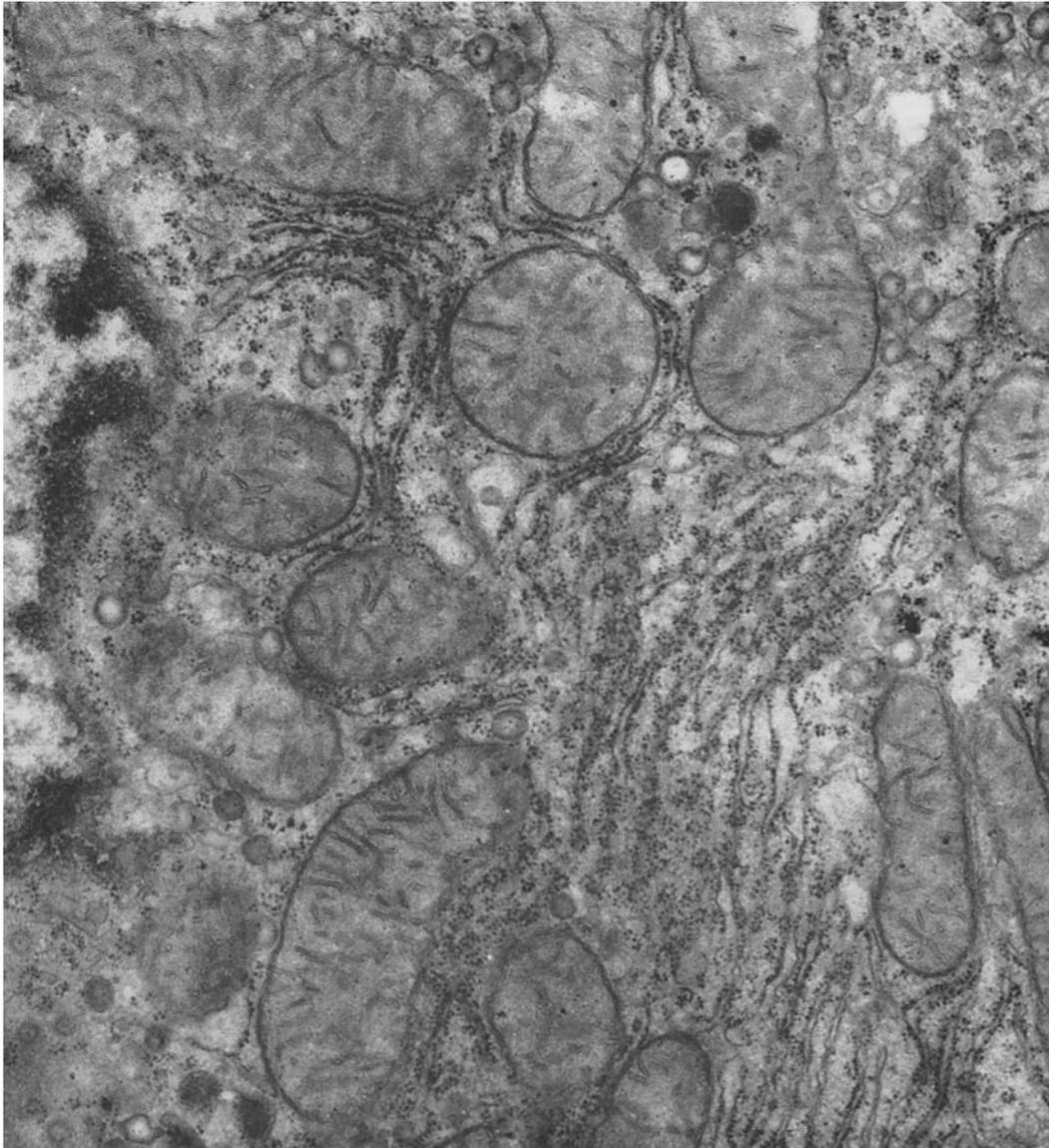


FIGURE 10 Parenchymal cell from a C3H mouse liver 7 hours after partial hepatectomy. Fixed in glutaraldehyde and refixed in osmium tetroxide, embedded in Epon, and stained with lead citrate. Note scarcity of glycogen particles. $\times 29,000$.

cytoplasmic bodies is revealed (Trotter, data unpublished). That fat can enter hepatic parenchymal cells by the process of pinocytosis has been demonstrated (32, 33). If the effects of partial hepatectomy are essentially an exaggeration of those of starvation, and fat is mobilized into the liver, the vesicle in which each of the cytoplasmic

bodies is located may represent a pinocytotic vesicle formed as the small, lipid-containing body enters the parenchymal cell. The observation that those bodies which are found in sinusoids and in the spaces of Disse are not located within vesicles, whereas those within hepatic parenchymal cells are, might tend to support this hypothesis; how-

ever, the number of electron-opaque bodies present in the sinusoids and the spaces of Disse at 3½ hours after partial hepatectomy is very small compared with the number present within the hepatic parenchymal cells. Thus, formation of pinocytotic vesicles, if it does take place, probably must occur earlier than any of the intervals after partial hepatectomy which were examined in this study. Examination at intervals as early as 10, 20, 40, 60, and 120 minutes after operation has been undertaken in order to ascertain whether any appreciable amount of pinocytosis can be observed.

It is, as previously mentioned, equally possible that the cytoplasmic bodies observed in regenerating liver at early intervals after partial hepatectomy represent an increased rate of *de novo* synthesis of lipid-containing bodies for export. It has been shown (13, 34, 35) that, soon after partial hepatectomy, there occurs a decrease in the total plasma protein content of the blood. Most of the proteins which are components of plasma lipoprotein are, under normal conditions, synthesized in the liver (13). If the cytoplasmic bodies of the regenerating liver are lipoprotein, it might be suggested that they are formed in the hepatic parenchymal cells to replace plasma lipoprotein lost after partial hepatectomy. It does not seem likely that the cytoplasmic bodies have any function in restoring the level of albumin in the blood, since the number of cytoplasmic bodies begins to decrease as early as 16 hours after partial hepatectomy and since the serum albumin content remains low until at least 5 days (35) after operation. The plasma globulin content, however, begins to rise 24 hours after operation (13, 34, 35). This increase does coincide with the decrease in number of cytoplasmic bodies observed in regenerating liver. It may be that the cytoplasmic bodies observed during the early hours after partial hepatectomy result from an increased *de novo* synthesis of plasma globulins by the liver, or an increase in the amount of globulin being exported. If this is true, it might be possible to find an increased number of these bodies in the sinusoids and in the spaces of Disse at 16 to 24 hours after partial hepatectomy when they disappear from the cytoplasm of the hepatic parenchyma. From the observations made in this study, this does not appear to be the case; however, additional work in this area is in progress and the possibility that the bodies contain plasma globulins for export

cannot be ruled out until more information is gathered.

The process by which lipoproteins are secreted from the liver into the circulation, *via* the hepatic sinusoid, is thought to involve the endoplasmic reticulum (36, 37). It has also been suggested that the endoplasmic reticulum may be concerned with lipid synthesis (38) and glycogenolysis (39). It will be recalled that, in regenerating liver, the decrease in glycogen content coincides with the increase in the number of cytoplasmic bodies in the hepatic parenchyma. Thus, if the above observations can be applied to the problem of liver regenerating after partial hepatectomy, the vesicles surrounding the cytoplasmic bodies may be part of the agranular endoplasmic reticulum assisting in the export of lipoprotein, synthesis of hepatic lipids, and/or glycogen depletion.

Whether the cytoplasmic bodies of regenerating liver are synthesized within the cell or whether they enter the cell from the blood stream, it does seem that they are somehow related to the large fat droplets which are always observed to accumulate at later postoperative intervals when the number of cytoplasmic bodies begins to decrease. Fat droplets of various sizes have been observed in contact with one another at all intervals after partial hepatectomy and in contact with the cytoplasmic bodies at the earlier intervals. It is suggested that the fat droplets may grow in size by a process of coalescing with one another and that the cytoplasmic bodies may also be incorporated into the fat droplets. At 48 hours after partial hepatectomy, the fat content of regenerating liver begins to decrease. During the period of fat breakdown (48 to 72 hours), no minute cytoplasmic bodies are observed; thus, it does not appear that fat breakdown is simply the reverse process of fat accumulation. It is known that lipid oxidation is carried on principally by mitochondria (40), and mitochondria have been observed in close morphological association with fat droplets (41 and 42). Since fat droplets in regenerating liver are observed in association with mitochondria, it is suggested that this is an observation of lipid oxidation. It has been noted that the cytoplasmic bodies are also occasionally associated with mitochondria. Since the cytoplasmic bodies are randomly distributed throughout the cell, there is perhaps no significance to this association. However, it may be that the cytoplasmic bodies are also oxidized by mitochondria.

This work was supported by grant AM-06558 from the National Institutes of Arthritis and Metabolic Diseases, United States Public Health Service, by grant H-6465 from the National Heart Institute, United States Public Health Service and administered by Dr. W. M. Copenhaver, and by grant IN-

45A from the American Cancer Society, allocated by Dr. J. Walter Wilson. The author wishes to thank Dr. Elizabeth H. Leduc for the facilities she so generously provided during a part of this study.

Received for publication, June 20, 1963.

REFERENCES

1. FISHBACK, F. C., A morphological study of regeneration of the liver after partial removal, *Arch. Path.*, 1929, **7**, 955.
2. COLLIP, J. B., KUTZ, R. L., LONG, C. N. H., THONSON, D. L., TOBY, G., and SELYE, H., Acute fatty liver following partial hepatectomy, *Canad. Med. Assn. J.*, 1935, **33**, 689, Abstract.
3. BRUES, A. M., DRURY, D. R., and BRUES, M. C., A quantitative study of cell growth in regenerating liver, *Arch. Path.*, 1936, **22**, 658.
4. LUDEWIG, S., MINOR, G. R., and HORTENSTINE, J. C., Lipid distribution in rat liver after partial hepatectomy, *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 158.
5. STOWELL, R. E., Nucleic acids and cytologic changes in regenerating rat liver, *Arch. Path.*, 1948, **46**, 164.
6. SZEGO, C. M., and ROBERTS, S., The influence of ovariectomy on the chemical composition of regenerating rat liver, *J. Biol. Chem.*, 1949, **178**, 827.
7. PRICE, J. M., and LAIRD, A. K., A comparison of the intracellular composition of regenerating liver and induced liver tumors, *Cancer Research*, 1950, **10**, 650.
8. ATERMAN, K., Some local factors in the restoration of the rat's liver after partial hepatectomy I. Glycogen; the Golgi apparatus; sinusoidal cells; the basement membrane of the sinusoids, *Arch. Path.*, 1952, **53**, 197.
9. HARKNESS, R. D., Changes in the liver of the rat after partial hepatectomy, *J. Physiol., London*, 1952, **117**, 267.
10. YOKOYAMA, H. O., WILSON, M. E., TSUBOI, K. K., and STOWELL, R. E., Regeneration of mouse liver after partial hepatectomy, *Cancer Research*, 1953, **13**, 80.
11. WILSON, M. E., STOWELL, R. E., YOKOYAMA, H. O., and TSUBOI, K. K., Cytological changes in regenerating mouse liver, *Cancer Research*, 1953, **13**, 86.
12. TSUBOI, K. K., YOKOYAMA, H. O., STOWELL, R. E., and WILSON, M. E., The chemical composition of regenerating mouse liver, *Arch. Biochem. and Biophys.*, 1954, **48**, 275.
13. GLINOS, A. D., The mechanism of liver growth and regeneration, in *The Chemical Basis of Development*, (W. D. McElroy and B. Glass, editors), Baltimore, Maryland, The Johns Hopkins Press, 1958, 813.
14. TAKAHASHI, T., An electron-microscope study on regenerating rat liver induced by partial hepatectomy, *Sapporo Med. J.*, 1960, **18**, 27.
15. BERNHARD, W., HAGUENAU, F., GAUTIN, A., and OBERLING, CH. La structure submicroscopique des elements basophiles cytoplasmiques dans le foie, le pancreas, et les glandes salivaires, *Z. Zellforsch. u. mikr. Anat.*, 1952, **37**, 281.
16. ROUILLER, C., and BERNHARD, W. "Microbodies" and the problem of mitochondrial regeneration in liver cells, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 355.
17. HIGGINS, G. M., and ANDERSON, R. M., Experimental pathology of the liver I. Restoration of the liver of the white rat following partial surgical removal, *Arch. Path.*, 1931, **12**, 186.
18. BARNUM, C. P., JARDETSKY, C. D., and HALBERG, F., Time relations among metabolic and morphologic 24-hour changes in mouse liver, *Am. J. Physiol.*, 1958, **195**, 301.
19. JAFFE, J. J., Diurnal mitotic periodicity in regenerating rat liver, *Anat. Rec.*, 1954, **120**, 935.
20. DALTON, A. J. and ZEIGEL, R. F., A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 409.
21. LUFT, J. H., Permanganate—a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
22. LEDUC, E. H., 1962, personal communication.
23. REYNOLDS, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.
24. SABATINI, D. D., BENSCH, K., and BARNETT, R. J., Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation, *J. Cell Biol.*, 1963, **17**, 19.
25. FAWCETT, D. W., Observations on the cytology and electron microscopy of hepatic cells, *J. Nat. Cancer Inst.*, 1955, **15**, 1475.
26. PORTER, K. R., and BRUNI, C., An electron

- microscope study of the early effects of 3'-ME-DAB on rat liver cells, *Cancer Research*, 1959, **19**, 997.
27. DAEMS, W. T., The micro-anatomy of the smallest biliary pathways in mouse liver tissue, *Acta Anat.*, 1961, **46**, 1.
 28. MILLONIG, G., and PORTER, K. R., Structural elements of rat liver cells involved in glycogen metabolism, in *Proc. European Regional Conf. Electron Micr., Delft.*, 1960, **2**, 655.
 29. CASLEY-SMITH, J. R., The identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals, *J. Cell Biol.*, 1962, **15**, 259.
 30. WASSERMANN, F., and McDONALD, T. F., Electron microscopic study of adipose tissue (fat organs) with special reference to the transport of lipids between blood and fat cells, *Z. Zellforsch. u. mikr. Anat.*, 1963, **59**, 326.
 31. HARKNESS, R. D., Regeneration of liver, *Brit. Med. Bull.*, 1957, **13**, 87.
 32. ASHWORTH, C. T., STEMBRIDGE, V. A., and SANDERS, E., Lipid absorption, transport and hepatic assimilation studied with electron microscopy, *Am. J. Physiol.*, 1960, **198**, 1326.
 33. PARKS, H. F., On the uptake of chylomicrons by hepatic cells of micc, *Anat. Rec.*, 1962, **142**, 320, Abstract.
 34. CHANUTIN, A., HORTENSTINE, J. C., COLE, W. S., and LUDEWIG, S., Blood plasma proteins in rats following partial hepatectomy and laparotomy, *J. Biol. Chem.*, 1938, **123**, 247.
 35. ALIVISATOS, S. G. A., STERN, K., SAVICH, B., and LUKACS, L., Serum proteins of rats after partial hepatectomy, *Proc. Soc. Exp. Biol. and Med.*, 1960, **103**, 465.
 36. REES, K. R., and SHOTLANDER, V. L., Fat accumulation in acute liver injury, *Proc. Roy. Soc. London, Series B*, 1963, **157**, 517.
 37. RECKNAGEL, R. O., and LOMBARDI, B., Studies of biochemical changes in subcellular particles of rat liver and their relationship to a new hypothesis regarding the pathogenesis of carbon tetrachloride fat accumulation, *J. Biol. Chem.*, 1961, **236**, 564.
 38. GREEN, D. E., The synthesis of fat, *Sc. Am.*, 1960, **202**, 46.
 39. PETERS, V. B., DEMBITZER, H. M., KELLY, G. W., and BARUCH, E., Ergastoplasmic changes associated with glycogenolysis, in *Proceedings of the 5th International Congress for Electron Microscopy* (S. S. Breese, Jr., editor), New York, Academic Press, Inc., 1962, **2**, TT-7.
 40. WAKIL, S. J., Lipid metabolism, *Ann. Rev. Biochem.*, 1962, **31**, 369.
 41. PALADE, G. E., and SCHIDLowsky, G., Functional association of mitochondria and lipide inclusions, *Anat. Rec.*, 1958, **130**, 352, Abstract.
 42. PALADE, G. E., Functional changes in the structure of cell components, in *Subcellular Particles*, (T. Hayashi, editor), New York, The Roland Press Company, 1959, 64.